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Research paper

Effect of *Lactobacillus brevis* ATCC 8287 as a feeding supplement on the performance and immune function of piglets

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ABSTRACT

Lactobacillus brevis ATCC 8287, a surface (S-layer) strain, possesses a variety of functional properties that make it both a potential probiotic and a good vaccine vector candidate. With this in mind, our aim was to study the survival of *L. brevis* in the porcine gut and investigate the effect of this strain on the growth and immune function of recently weaned piglets during a feeding trial. For this, 20 piglets were divided evenly into a treatment and a control group. Piglets in the treatment group were fed *L. brevis* cells (1×10^{10}) daily for three weeks, whereas those in the control group were provided an equivalent amount of probiotic-free placebo. For assessing the impact of *L. brevis* supplementation during the feeding trial, health status and weight gain of the piglets were monitored, pre- and post-trial samples of serum and feces were obtained, and specimens of the small and large intestinal mucosa and digesta were collected at slaughter. The results we obtained indicated that *L. brevis*-supplemented feeding induced a non-significant increase in piglet body weight and caused no change in the morphology of the intestinal mucosa. *L. brevis* cells were found to localize mainly in the large intestine, but they could not be isolated from feces. To a lesser extent, *L. brevis* was detected in the small intestine, although there was no specific attachment to the Peyer's patches. Changes in total serum IgG and IgA concentrations were not caused by supplemented *L. brevis* and no measurable rise in *L. brevis*-specific IgG was observed. However, analysis of cytokine gene expression in intestinal mucosa revealed downregulation of TGF-β1 in the ileum and upregulation of IL-6 in the cecum in the *L. brevis*-supplemented group. Based on the results from this study, we conclude that whereas *L. brevis* appears to have some intestinal immunomodulatory effects, the ability of this strain to survive and colonize within the porcine gut appears to be limited.

1. Introduction

Lactobacilli are important indigenous members of the gut microbiota in both humans and animals and their use in food processing and preservation has a long tradition and history. Several species, especially those among the *Lactobacillus* genera, have beneficial properties and are

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considered important for mammalian health (Nousiainen et al., 2004; Bosi and Trevisi, 2010). Additionally, in the last decade, there has been an increased interest in the use of lactobacilli as delivery vectors, particularly for the development of mucosal-administered vaccines (Bermudez-Humaran et al., 2011; Wells, 2011a). Many of the *Lactobacillus* species fulfill most criteria considered important for bacteria being used as probiotics and/or whole-cell vaccines. For instance, these include surviving the harsh conditions of the gastrointestinal (GI) tract, adhering to host intestinal cells, colonizing the gut, competitively excluding pathogens, modulating host immune responses, and a safety profile status (Dunne et al., 2001; Mohamadzadeh et al., 2008). In addition, when used in vaccine development, the comparatively lower intrinsic immunogenicity of lactobacilli than that found with attenuated pathogenic strains is regarded as a favorable property (Pouwels et al., 1998; Wells and Mercenier, 2008). On the other hand, some lactobacilli can modulate host immune responses, and this adjuvant-like property can also be a benefit in *Lactobacillus*-vectored vaccines (Licciardi and Tang, 2011; Wells, 2011b).

Among lactobacilli, there are some species that have surface layers (S-layers), which consist of crystalline bidimensional arrays of either glycosylated or non-glycosylated protein subunits, and represent the outermost coating of the bacterial cell wall. Interestingly, S-layer proteins from various *Lactobacillus* hosts have been found to mediate bacterial adhesion to epithelial cells, intestinal mucus and extracellular matrix (ECM) proteins, which in some instances inhibit pathogen binding to host tissues (Mobili et al., 2010). Moreover, a protective function of the S-layer coating has been suggested when cells become exposed to bile salts, pancreatic extract, pH fluctuations and thermal shocks (Mobili et al., 2010). Utilization of S-layer proteins for the surface display of foreign antigenic epitopes has been explored (Ilk et al., 2011), and represent an interesting approach in the development of whole-cell vaccines, since the production of the epitope in each S-layer subunit as part of the overall lattice structure would enable bacteria with such chimeric S-layer lattices to display a large number (e.g., $\sim 5 \times 10^5$/bacterium) of antigenic molecules on their cell surface (Sleytr et al., 2007).

Lactobacillus brevis strains can be found in fermented foods and other environments, including the GI tract and feces of humans and other animals like pigs. *L. brevis*, a close relative of *Lactobacillus plantarum* (Kant et al., 2011), is considered safe for human and animal consumption and thus accredited with a QPS (Qualified Presumption of Safety) status by the European Food Safety Authority. A variety of *L. brevis* strains have been reported in the literature to have several probiotic properties, including tolerance to acid and bile (Rönkä et al., 2003), the ability to adhere to intestinal cells, mucus, and human intestinal blood type-A antigen (Ouwehand et al., 2001; Rönkä et al., 2003; Uchida et al., 2006), survivability through the GI tract (Rönkä et al., 2003), bacteriocin production (Klaenhammer, 1993; Wada et al., 2009), antimicrobial activity against intestinal pathogens (Hillman and Fox, 1994; Martin et al., 2009), and enhancement of intestinal barrier function (Ueno et al., 2011). Despite this, however, there are only a few reports of studies that describe *in vivo* animal trials involving *L. brevis*. For example, the *L. brevis* La-11 strain, a fecal isolate from pigs, was observed to have an ameliorating effect on neonatal diarrhea in calves when administered orally as microencapsulated spray dried formula (Qi et al., 2011). Likewise, when *L. brevis* 1E1, which had been isolated from porcine esophagus, was administered as a milk supplement in piglets, it was shown to have an immunomodulating impact in the intestine and to reduce the digesta coliform counts, ultimately resulting in enhanced pig growth (Brown et al., 2006; Gebert et al., 2011). Moreover, when the mouse-isolated *L. brevis* ML12 was administered orally, it demonstrated adjuvant effects in mice immunized i.p. with trinitrophenyl-conjugated chicken gamma globulin (TNP-CGG) and also modulated cytokine responses in mice immunized with Chikungunya virus (Maassen et al., 2000). Lastly, *L. brevis* KB290, a strain isolated from the Japanese sugugi pickle, exhibited an increased production of IFN-α in humans after being administered orally for four weeks (Kishi et al., 1996).

The *L. brevis* ATCC 8287 strain was recovered originally from fermented green olives and has been shown previously to possess numerous probiotic properties, including a good adhesion capacity (Rönkä et al., 2003). For example, based on the results from adhesion studies performed *in vitro*, this strain has binding affinity to the human Caco-2 and Int-407 (Hynönen et al., 2002; Rönkä et al., 2003), porcine IPEC-1 (Hynönen, personal communication) intestinal cell lines, porcine-isolated intestinal epithelial cells (Jakava-Viljanen and Palva, 2007), as well as to different ECM proteins, including collagen, fibrinogen and laminin (Rönkä et al., 2003; Jakava-Viljanen and Palva, 2007). *L. brevis* ATCC 8287 is an S-layer containing strain, and the corresponding *slpA* gene product (SlpA) has been characterized, including the receptor-binding, self-assembly, and cell wall binding domains (Vidgrén et al., 1992; Åvall-Jääskeläinen et al., 2003, 2008). In addition, several surface-accessible residues in the monomeric protein structure have been localized (Vilén et al., 2009). Moreover, the SlpA protein can mediate cellular adhesion to intestinal cell lines and ECM components *in vitro* (Åvall-Jääskeläinen et al., 2003; de Leeuw et al., 2006). Furthermore, because an 11-residue long epitope of the human c-myc proto-oncogene could be produced throughout the S-layer lattice structure of this particular strain (Åvall-Jääskeläinen et al., 2002), *L. brevis* ATCC 8287 is viewed as an attractive candidate for the design of an oral vaccine vector to deliver a large amount of antigens to the immune system. With this purpose in mind, we have investigated the potential effects of *L. brevis* ATCC 8287 as a feeding supplement on the performance and immune function in piglets as a means to evaluate the suitability of this strain for use as both a viable probiotic and a recombinant vaccine vector in swine.

2. Materials and methods

2.1. Animal trial

Twenty commercially-bred piglets of both genders (8 Finnish Landrace, 4 Finnish Yorkshire, and 8 backcrossing of